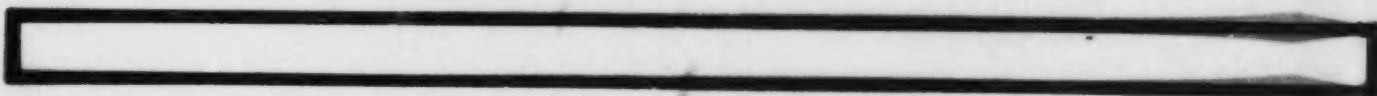


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Biotransformation of Selected
Organic Pollutants in Ground Water

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CHAPTER 17

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JOURNAL ARTICLE

Biotransformation of Selected Organic Pollutants in Ground Water

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Widespread contamination of ground water by a variety of synthetic organic compounds has created a need for information about the biotransformation of these contaminants in aquifers and associated regions of the unsaturated subsurface. Uncontaminated samples of the subsurface were taken from positions immediately above and below the water table at Pickett, OK, and Fort Polk, LA. Selected organic pollutants were added aseptically to the samples at an initial concentration of 1 mg/liter water. No degradation of 1,1,1-trichloroethane, chloroform, or 1,1-dichloroethane was detected in any of the samples. Toluene and styrene was degraded slowly in samples from both positions at both sites. Chlorobenzene was degraded in samples from both positions at Pickett, but no degradation was detected in samples from Fort Polk. There was no detectable degradation of these three compounds in autoclaved samples. Tri- and tetrachloroethylene was degraded very slowly in some of the samples. These rates, however, were the same magnitude as published rates of chemical hydrolysis. Microbes in the deeper subsurface can degrade some, but not all, of the organic pollutants commonly encountered in ground water.

INTRODUCTION

Ground water is a vital natural resource. Approximately one-half of the population of the United States depends on ground water for its domestic water supply. One-fifth of the population is served by rural domestic wells (USEPA 1977), which are particularly vulnerable to undetected contamination. Many organic pollutants commonly found in ground water have no taste or odor at concentrations that entail serious health risks. Private wells are rarely examined for organic pollutants because the analysis for these pollutants is expensive (Council on Environmental Quality 1981).

Pollution by organic compounds is widespread in ground water. At least one state in each of the 10 EPA regions has reported significant pollution of ground water by organic compounds (Council on Environmental Quality 1981). An extensive survey of wells in New Jersey reveals that ground water in that state is at least as polluted by organic contaminants as surface water (Page 1981). Volatile organic compounds have been detected in 45% of the large public water supplies which distribute ground water (USEPA 1982). Information on biotransformations of organic pollutants in ground water is needed to manage this resource intelligently, to prevent future contamination, and to identify economical remedies to existing pollution.

The literature on biodegradation of organic compounds in surface soils is extensive, but this information cannot be extrapolated directly to the deeper subsurface. There is a modest, but useful, literature on biotransformations of organic compounds

in deep saline aquifers, developed through the interest of the United States Geological Survey in deep-well disposal of industrial wastes (Leenheer et al. 1976; Ehrlich et al. 1979). Unfortunately, useful information is scanty on biotransformations of organic contaminants in shallower aquifers containing potable water (Roberts et al. 1980; Schneider et al. 1981).

This study is a preliminary assessment of the impact of biological processes on the fate of several important organic contaminants in the subsurface environment. The fate of 1,1-dichloroethane, chloroform, 1,1,1-trichloroethane, trichloroethylene, tetrachloroethylene, toluene, chlorobenzene, and styrene was determined in authentic subsurface materials that were uncontaminated by surface microbes. The chlorinated aliphatic hydrocarbons examined in this study are important contaminants of ground water (USEPA 1982). The substituted benzenes are not found as frequently in ground water. However, they are degraded easily and were included in the study to define more precisely the degradative potential of the subsurface microflora. The fate of the organic compounds was studied in subsurface material from two sites with shallow water-table aquifers. Such aquifers should be the most vulnerable to pollution by organic contaminants. The organic compounds were added to the subsurface material at concentrations (600 to 800 $\mu\text{g/liter}$) that are characteristic of intensely polluted environments, and that are likely to provide a substantive threat to human health (Tucker 1981).

MATERIALS AND METHODS

Acquisition of Uncontaminated Samples of the Subsurface

Material was collected from positions immediately above and below the water table at sites in Pickett, OK, and Fort Polk, LA.

A modification of procedures described by Dunlap et al. (1977) was used to acquire samples that were not contaminated with surface microbes. A bore hole of the desired depth was drilled with an auger. Then the auger was removed and the sample taken with an autoclaved thin-wall core barrel 9.5 cm i.d. x 45 cm long (Fig. 1). The core was extruded 3 to 5 cm, then broken off to produce an aseptic face. The remainder of the core was extruded past a device that pared away the outer 1.2 cm of core material (Fig. 2). The core material that was in contact with the core barrel and might be contaminated with surface microbes was discarded. The last 4 cm of the core was not extruded to preclude contamination from the extruder. At the Pickett site, a special, noncontaminating Teflon well was constructed to provide water from the same stratum of sand that was sampled by the core barrel.

Preparation of Microcosms of the Subsurface Environment

A small amount of sterile water was mixed into the uncontaminated subsurface material until it became a slurry; then the slurry was transferred to 35-ml screwcap test tubes (Fig. 3). Space was left for approximately 1 ml of a dose solution. To prepare dose solutions, water from the Teflon well was filter-sterilized, then aseptically dosed with the organic chemicals and stirred in a sealed container until all compounds were dissolved. The remaining space in the test tubes was filled with this

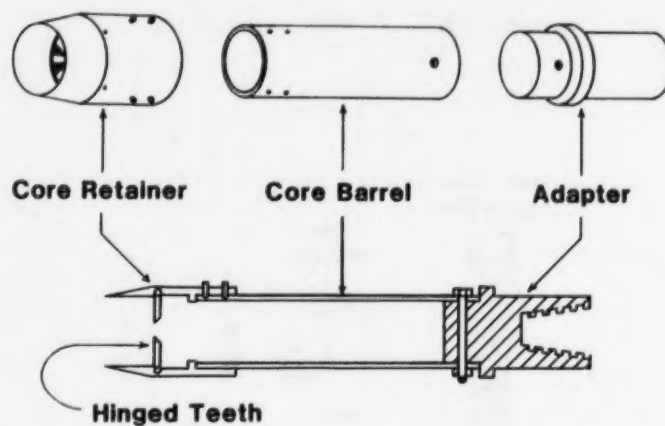


FIG. 1. Coring device.

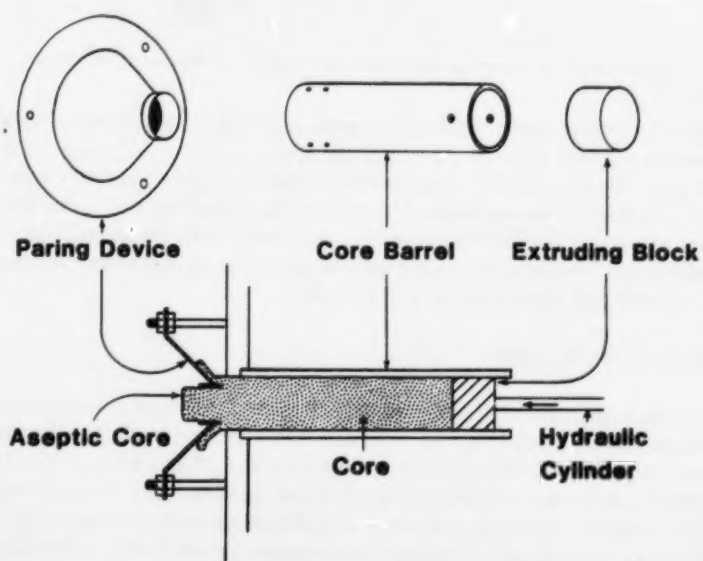


FIG. 2. Device to recover cored material aseptically.

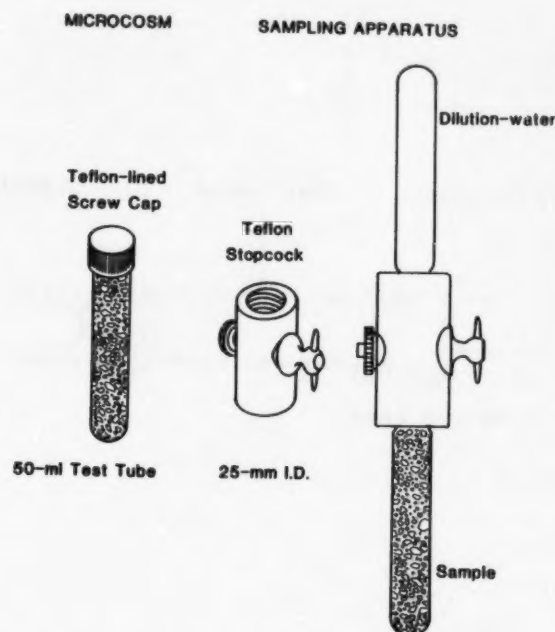


FIG. 3. Microcosm of the subsurface environment and sampling device for the microcosm.

dosed well water so that no head space remained. The mouth of the tube was wiped clean with a sterile piece of paper, and the tube sealed with a 25-mm Teflon-lined screw cap. The dose solution then was dispersed throughout the slurry with a vortex mixer. The initial concentrations of each organic compound was 1 mg/liter of pore water. To provide an abiotic control, a portion of the subsurface material was autoclaved prior to being dispensed into the test tubes and amended with chemicals. The material was autoclaved at 121 C for 4 h.

Analysis of Microcosms

The microcosms were sampled by diluting the pore water in a way that prevented loss of volatile compounds (Fig. 3). The cap was removed and the microcosm was screwed into one end of a large Teflon stopcock with a 10-mm bore. A 35-ml test tube filled with water was screwed into the other end. Subsequently, the cock was opened and the contents of the two tubes were mixed together with a vortex mixer. Finally, the cock was closed, and the tubes were separated, sealed with Teflon-lined screw caps, and centrifuged gently until the diluted pore water was clear. Microcosms were analyzed after 0, 1, 3, 9, and 27 wk of incubation in the dark at 17 C. Due to a shortage of material, microcosms made up with samples from below the water table at Fort Polk were incubated only 0, 1, 3, and 9 wk. At each time period, at least two microcosms plus a microcosm made up with autoclaved material were analyzed.

The diluted pore water was analyzed essentially in accordance with EPA protocol Method #601 (USEPA 1979). A 10-ml sample was purged with N_2 at 40 ml min^{-1} for 12 min, using a Hewlett Packard 7675A Purge and Trap Unit. Volatile organic compounds were collected on a Tenax resin trap, then thermally desorbed into a gas chromatograph for analysis. The limit of detection was $<0.20 \mu g/liter$ for the aliphatic hydrocarbons and $<0.08 \mu g/liter$ for the aromatic hydrocarbons.

Characterization of Samples

Sand, silt, and clay content were determined by sedimentation according to procedure D422-54T prescribed by ASTM (1958). Total organic carbon in soil was determined by combustion with a Leco WR-12 Carbon Analyzer (Leco Corp., St. Joseph, MO).

RESULTS AND DISCUSSION

The sampling sites at Fort Polk, LA, and Pickett, OK, are fairly typical of shallow, water-table aquifers in the south-central region of the United States (Table 1). All samples were aerobic when collected. The materials differed from surface soils primarily in their almost total lack of structure, and in their low organic-carbon content.

TABLE 1. *Characteristics of samples of the deeper subsurface environment*

Sample	Depth (meters)	<i>In situ</i> Temperature	pH	Total Organic C (% dry wt)	Sand	Silt (%dry wt)	Clay
Fort Polk, LA							
Above water table	2.1	20 C	4.3	0.015	91	1.8	7.3
Below water table	3.6		4.2	0.014	90	3.2	6.1
Pickett, OK							
Above water table	3.6	17 C	7.8	0.02	91	4.0	5.1
Below water table	4.8		5.7	0.02	91	4.2	4.8

Degradation of Organic Pollutants by Subsurface Microbes

The fate of selected organic pollutants in samples from the subsurface is presented in Table 2. The experimental procedure generally could detect rates of degradation in excess of 1-3%/wk. The minimum detectable rate was much higher in autoclaved material from below the water table at Fort Polk. This was due to a smaller number of samples and a shorter interval of incubation, and not to greater variability in this material.

There was no evidence of degradation of 1,1-dichloroethane, chloroform, or 1,1,1-trichloroethane. These halogenated aliphatic hydrocarbons generally are considered to be resistant to biodegradation (McConnell et al. 1975), although they have been reported to biodegrade aerobically in at least one system (Tabak et al. 1981), and the biodegradation of chloroform has been reported in anaerobic systems (Bouwer et

TABLE 2. Degradation of organic pollutants in samples of the deeper subsurface environment

Compound	Autoclaved	Pickett, OK		Fort Polk, LA	
		Above ^a	Below ^a	Above ^a	Below ^a
		% initial concentration ^b degraded per wk			
1,1-Dichloroethane	No	<1.2 ^c	<1.9 ^c	<1.2 ^c	<2.6 ^c
	Yes	<1.5	<2.9	<1.6	<25
Chloroform	No	<1.0	<2.0	<1.1	<2.8
	Yes	<1.4	<2.6	<1.4	<21
1,1,1-Trichloroethane	No	<1.1	<1.8	<1.2	<3.2
	Yes	<1.2	<3.2	<1.6	<35
Trichloroethylene	No	+1.3 ± 1.1	<1.3	<1.2	<2.3
	Yes	<1.5	<2.2	<1.4	<26
Tetrachloroethylene	No	+1.1 ± 0.8	+1.3 ± 1.0	+0.9 ± 0.8	<1.8
	Yes	<1.2	<2.0	<1.8	<16
Toluene	No	+0.9 ± 0.8	+2.4 ± 1.0	+2.5 ± 0.9	+3.2 ± 3.2
	Yes	<1.4	<1.4	<1.2	<12
Chlorobenzene	No	+0.9 ± 0.8	+1.9 ± 1.3	+0.6 ± 1.0	-0.2 ± 1.9
	Yes	<1.5	<2.4	<1.5	<13
Styrene	No	+2.3 ± 0.7	+4.3 ± 3.6	+3.8 ± 1.1	+12.0 ± 5.6
	Yes	<0.8	<2.5	<1.9	<12

^aSamples taken immediately above and below the water table.^b600-800 µg/liter.^cSlope of regression line ± 95% confidence interval on the slope.

al. 1981). Their persistence in this study is consistent with their observed persistence in ground water (Zoeteman et al. 1981).

Degradation of trichloroethylene and tetrachloroethylene was detectable in some of the subsurface materials. However, it is likely that degradation was produced by abiotic processes. Dilling et al. (1975) reported abiotic degradation rates for trichloroethylene and tetrachloroethylene (1.6 and 2.0% wk⁻¹, respectively) which are close to the rates determined in this study.

Toluene and styrene degraded slowly in all four samples. There was no detectable degradation of these compounds in autoclaved material. Unfortunately, the minimum detectable rate of degradation of toluene in two of the autoclaved subsurface samples was too high to determine whether degradation in the nonautoclaved samples was reduced by autoclaving. However, degradation of toluene in two samples, and of styrene in all four samples, was clearly reduced by autoclaving and presumably can be attributed to biological activity.

Chlorobenzene degraded slowly in material from the Pickett site; there was no detectable degradation in material from the Fort Polk site. Again, the minimum detectable rate of degradation in the autoclaved samples was too high to distinguish between biodegradation and abiotic degradation in the nonautoclaved samples.

Correspondence between Rates of Biodegradation and Microbial Numbers

The microbes indigenous to the material from below the water table at Pickett, OK, have been characterized by Ghiorse and Balkwill (1981). The predominant forms were small, coccoidal bacteria generally $<0.8 \mu\text{m}$ diam. No eucaryotic forms were detected. The numbers of these subsurface microbes were surprisingly high. They found $5 \times 10^6/\text{g}$ dry material by direct count using epifluorescence microscopy, and counted 3.1×10^6 colony-forming units (CFU's)/g on a nutrient-poor medium made from soil extract and 2.5×10^6 CFU's/g on a dilute complex medium.

Wallis et al. (1979) have shown that organic matter in shallow ground water under a forest in Canada, when discharged through springs to a stream, was taken up and used by the benthic organisms. Clearly, infiltrating ground water was not stripped entirely of metabolizable compounds. He found densities of planktonic bacteria in the ground water ($10^5/\text{ml}$ by epifluorescence microscopy) roughly equivalent to the densities reported by Ghiorse and Balkwill (1981). These organisms obviously are supported by the available organic compounds dissolved in infiltrating ground water.

If a significant fraction of the population were active against the pollutants, they could degrade the pollutants at an appreciable rate. A steady-state population of $5 \times 10^6/\text{g}$, each weighing $1 \times 10^{-14}\text{g}$, and having a rather conservative specific maintenance-value of $0.2/\text{wk}$ (Shields et al. 1973) would consume $0.01 \mu\text{g}$ organic compounds g^{-1} dry material week^{-1} . The subsurface material is 20% water. This corresponds to $50 \mu\text{g}$ organic compounds $\text{liter}^{-1} \text{week}^{-1}$.

The actual combined rate of degradation of toluene, chlorobenzene, and styrene in subsurface material from below the water table at Pickett, OK, was $60 \mu\text{g liter}^{-1} \text{week}^{-1}$. This close correspondence does not indicate that all the microbes in this material were active against at least one of the compounds, or that these compounds meet the entire energy requirements of the population. It does indicate that the observed density of organisms can account for the observed rate of degradation.

Oligotrophy in Ground-Water Microbes

The pore water in material from below the water table at Pickett contained only $2.4 \text{ mg organic matter/liter}$ (Bengtsson 1981). The bacteria indigenous to this environment must, of necessity, be oligotrophs. Kuznetsov et al. (1979) observed that oligotrophic bacteria from surface water frequently fail to grow on rich, complex media. The bacteria indigenous to the deeper subsurface also fail to grow on complex media. Ghiorse and Balkwill (1981) counted 6×10^2 CFU/g in material from below the water table at Pickett on conventional peptone, yeast extract, glucose medium, and 3.1×10^6 CFU/g on the same medium diluted to 5% of its normal concentration. Similarly, Bengtsson (1981) counted only $5.4\text{--}6.4 \times 10^3$ CFU/g in four separate samples of this subsurface material using a conventional complex medium.

Apparently many oligotrophic bacteria from surface water fail to use organic compounds that are used readily by eutrophic forms. Ishida and Kadota (1981) described oligotrophic bacteria from a lake that would not take up radiolabeled glucose or acetate, although they did take up glycolate and many amino acids. Lucyszyn and Pritchard (1979) reported on an oligotrophic strain of *Pseudomonas* which also

failed to take up glucose. Subba-Rao et al. (1982) found that 2,4-dichlorophenoxyacetic acid was mineralized by organisms in sewage and water from an eutrophic pond, but not in water from a meso- or oligotrophic lake. Similarly, bis(2-ethylhexyl) phthalate was mineralized in water from the eutrophic pond, but not in water from the oligotrophic lake.

Such effects suggest that the bacteria of the deeper subsurface, being oligotrophs, will be active against a more limited range of organic compounds than are degraded in surface soil. Chlorobenzene is degraded readily in surface soil (Wilson et al. 1981), but there was no detectable degradation of chlorobenzene in subsurface material from Fort Polk, even though there was considerable activity against styrene and toluene. Similarly, Roberts et al. (1980) found that chlorobenzene did not degrade during passage of renovated municipal wastewater through an alluvial aquifer in San Francisco Bay. Wallis et al. (1979) found that uptake of radiolabeled glutamate per cell was one hundred to one thousand times slower in planktonic ground-water bacteria compared to bacteria in stream water.

Ecology of Bacteria in the Deeper Subsurface

The subsurface is a remarkably stable environment. In such an environment the bacteria may have evolved into the procaryotic equivalent of cave fish, which have given up traits useful only to the surface, such as sight, pigmentation, and the ability to swim actively, and instead have developed a slow metabolism to allow them to survive the long intervals between capture of prey, and a sensitive lateral-line system, which they use to hunt for food. In the presence of predators, populations of bacteria must grow at least as rapidly as they are being grazed, or they will be grazed out. The absence of predation from eucaryotic organisms may allow subsurface bacteria to select much lower growth rates and slower metabolisms. Because their food supply, dissolved organic matter in the soil water infiltrating from the surface, is fairly constant in supply and composition, there would be an opportunity for specialization in capture of metabolizable organic compounds from very dilute solution.

The next task is to identify the special characteristics of the subsurface that control the development of the microbes and the special adaptations made by the microbes of that particular environment.

DISCLAIMER NOTICE

Although the research described in this article has been funded by the United States Environmental Protection Agency through an in-house project at the Robert S. Kerr Environmental Research Laboratory, it has not been subjected to the Agency's required peer and policy review and, therefore, does not necessarily reflect the views of the Agency and no official endorsement should be inferred.

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